

Immune cell subset panel				
Antibody target	Clone	Fluorophore	Vendor	Catalog #
CD3	OKT3	Alexa 647	BioLegend	317312
CD4	A161A1	PE	BioLegend	357404
CD8	SK1	Alexa 700	BioLegend	344724
CD14	HCD14	BV421	BioLegend	325628
CD16	B73.1	PE-Cy7	BioLegend	360708
CD19	HIB19	PerCP-Cy5.5	BioLegend	302230
Live/dead dye		eFluor 506	Invitrogen	65-0866-14
Activation induced marker (AIM) panel				
Antibody target	Clone	Fluorophore	Vendor	Catalog #
CD4	OKT4	Alexa 488	BioLegend	317420
CD8	SK1	Alexa 700	BioLegend	344724
OX-40 (CD134)	ACT35	PE-Dazzle 594	BioLegend	350020
CD69	FN-50	PE	BioLegend	310906
4-1BB (CD137)	4B4-1	BV421	BioLegend	309820
CD14	HCD14	PerCP-Cy5.5	BioLegend	325622
CD16	B73.1	PerCP-Cy5.5	BioLegend	360712
CD19	HIB19	PerCP-Cy5.5	BioLegend	302230
Live/dead dye		eFluor 506	Invitrogen	65-0866-14
Intracellular cytokine stimulation (ICS)				
Antibody target	Clone	Fluorophore	Vendor	Catalog #
CD4	A161A1	PE	BioLegend	357404
CD8	SK1	Alexa 700	BioLegend	344724
CD14	HCD14	PerCP-Cy5.5	BioLegend	325622
CD16	B73.1	PerCP-Cy5.5	BioLegend	360712
CD19	HIB19	PerCP-Cy5.5	BioLegend	302230
IFN γ	4S.B3	Alexa 647	BioLegend	502516
TNF α	MAb11	Alexa 488	BioLegend	502915
IL-2	MQ1-17H12	BV421	BioLegend	500328
IL-4	MP4-25D2	APC-Cy7	BioLegend	500833
IL-10	JES3-9D7	PE-Dazzle 594	BioLegend	501426
Live/dead dye		eFluor 506	Invitrogen	65-0866-14
Tetramer panel				
Antibody target	Clone	Fluorophore	Vendor	Catalog #
CD8	SK1	PE-Cy7	eBioscience	25-0087-42
CD4	RPA-T4	PerCP-Cy5.5	BioLegend	300530
CD14	HCD14	PerCP-Cy5.5	BioLegend	325622
CD16	B73.1	PerCP-Cy5.5	BioLegend	360712
CD19	HIB19	PerCP-Cy5.5	BioLegend	302230
CCR7	G043H7	Alexa 488	BioLegend	353206
CD45RA	HI100	APC/Fire 750	BioLegend	304152
Streptavidin		PE	Invitrogen	S866
Streptavidin		APC	Invitrogen	S868
Streptavidin		BV421	BioLegend	405225
Streptavidin		PE-Dazzle 594	BioLegend	405247
Live/dead dye		eFluor 506	Invitrogen	65-0866-14

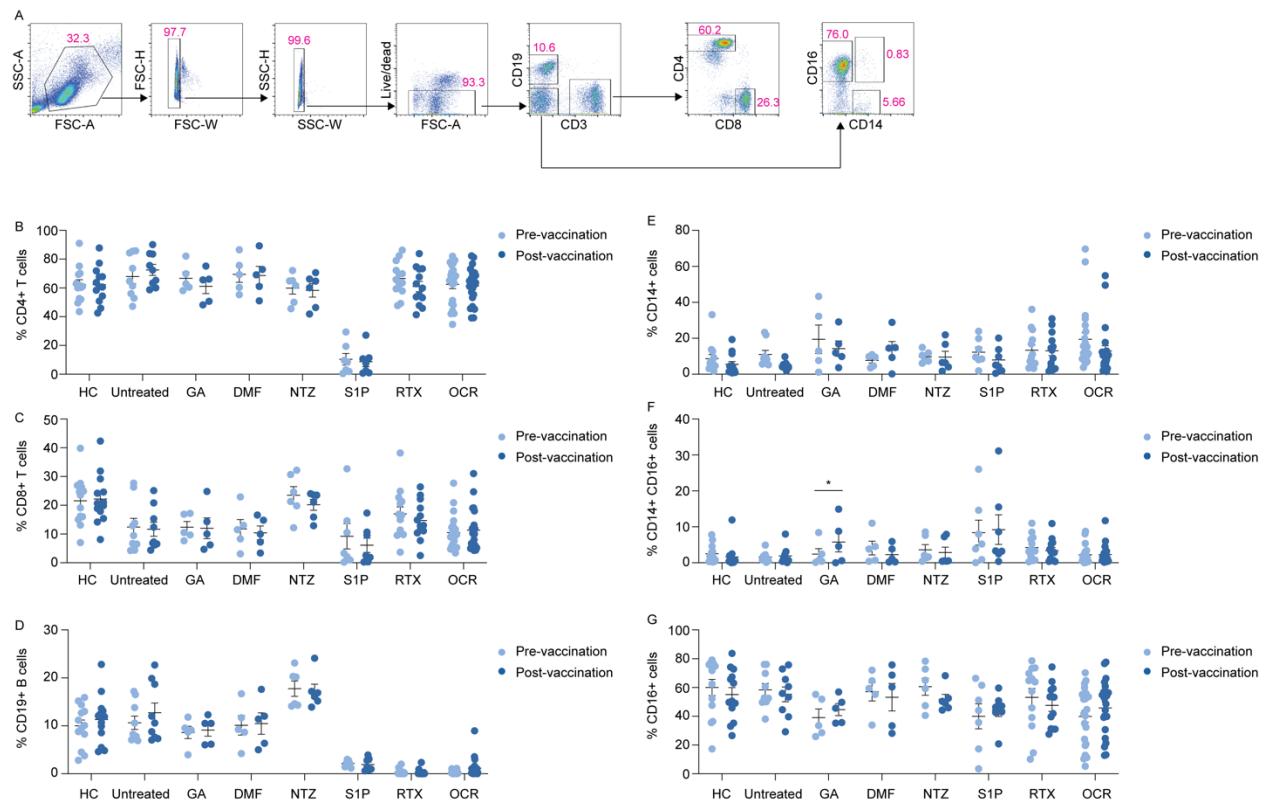
Supplemental Table 1. Flow cytometry panel overview. Antibodies labeled with the same fluorophore in a given panel were used to create a 'dump' channel for exclusion of the designated populations.

Tetramer panel 1			
Epitope	MHC I restriction	Fluorophore 1	Fluorophore 2
YLQPRTFLL	A*02:01	PE	APC
RLQSLQTYV	A*02:01	PE	BV421
VVFLHVTYV	A*02:01	PE	PE-Dazzle 594
SPRRARSVA	B*07:02	PE-Dazzle 594	BV421
APHGVVF	B*07:02	PE-Dazzle 594	APC
Tetramer panel 2			
Epitope	MHC I restriction	Fluorophore 1	Fluorophore 2
KCYGVSP	A*03:01	PE	APC
GVYFASTEK	A*03:01	PE	BV421
GVYFASTEK	A*11:01	PE	PE-Dazzle 594
GTHWFVTQR	A*11:01	PE-Dazzle 594	BV421
RLFRKSNLK	A*11:01	PE-Dazzle 594	APC
Tetramer panel 3			
Epitope	MHC I restriction	Fluorophore 1	Fluorophore 2
KCYGVSP	A*03:01	PE	APC
GVYFASTEK	A*03:01	PE	BV421
LTDEMIAQY	A*01:01	PE	PE-Dazzle 594

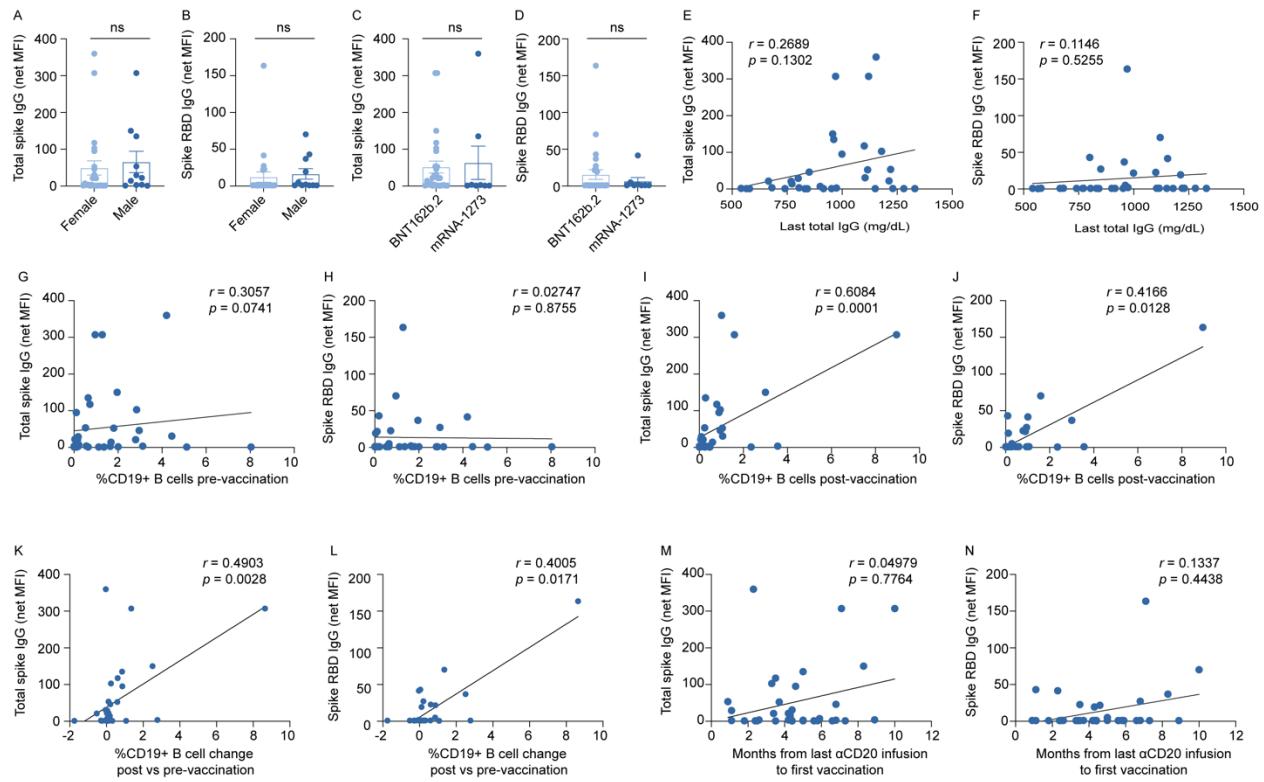
Supplemental Table 2. Overview of pMHC I tetramer panels. The epitope, MHC I restriction, and fluorophore combinations used for the indicated tetramer panels are shown.

Sample ID	Treatment	HLA restriction for tetramer staining		Tetramer panel
HCCOV001	HC	HLA-A*11:01		2
HCCOV003	HC	HLA-A*03:01	HLA-A*11:01	2
HCCOV009	HC	HLA-A*11:01		2
HCCOV011	HC	HLA-A*02:01	HLA-B*07:02	1
HCCOV015	HC	HLA-A*02:01		1
MSCOV020	None	HLA-A*03:01		2
MSCOV027	None	HLA-A*02:01	HLA-B*07:02	1
MSCOV059	None	HLA-A*02:01		1
MSCOV070	None	HLA-A*02:01		1
MSCOV082	None	HLA-A*03:01		2
MSCOV009	GA	HLA-B*07:02		1
MSCOV047	GA	HLA-B*07:02		1
MSCOV077	GA	HLA-B*07:02	HLA-A*11:01	1 & 2
MSCOV010	DMF	HLA-A*01:01		3
MSCOV021	DMF	HLA-A*03:01		2
MSCOV035	DMF	HLA-A*03:01	HLA-A*11:01	2
MSCOV038	DMF	HLA-A*03:01	HLA-A*01:01	3
MSCOV089	DMF	HLA-B*07:02	HLA-A*03:01	1 & 2
MSCOV037	NTZ	HLA-A*03:01	HLA-A*01:01	3
MSCOV039	NTZ	HLA-A*02:01		
MSCOV052	NTZ	HLA-A*02:01	HLA-B*07:02	HLA-A*03:01
MSCOV057	NTZ	HLA-A*03:01		2
MSCOV069	NTZ	HLA-B*07:02		1
MSCOV016	S1P	HLA-A*02:01		1
MSCOV050	S1P	HLA-B*07:02	HLA-A*03:01	1 & 2
MSCOV060	S1P	HLA-A*02:01	HLA-B*07:02	1
MSCOV072	S1P	HLA-A*01:01		3
MSCOV012	RTX	HLA-A*01:01		3
MSCOV025	RTX	HLA-B*07:02		2
MSCOV049	RTX	HLA-A*11:01		2
MSCOV074	RTX	HLA-A*02:01		1
MSCOV076	RTX	HLA-A*03:01	HLA-A*11:01	2
MSCOV084	RTX	HLA-A*02:01		1
MSCOV014	OCR	HLA-A*11:01		2
MSCOV022	OCR	HLA-B*07:02		1
MSCOV045	OCR	HLA-B*07:02		1
MSCOV056	OCR	HLA-A*02:01		1
MSCOV058	OCR	HLA-A*03:01		2
MSCOV064	OCR	HLA-A*03:01	HLA-A*01:01	3

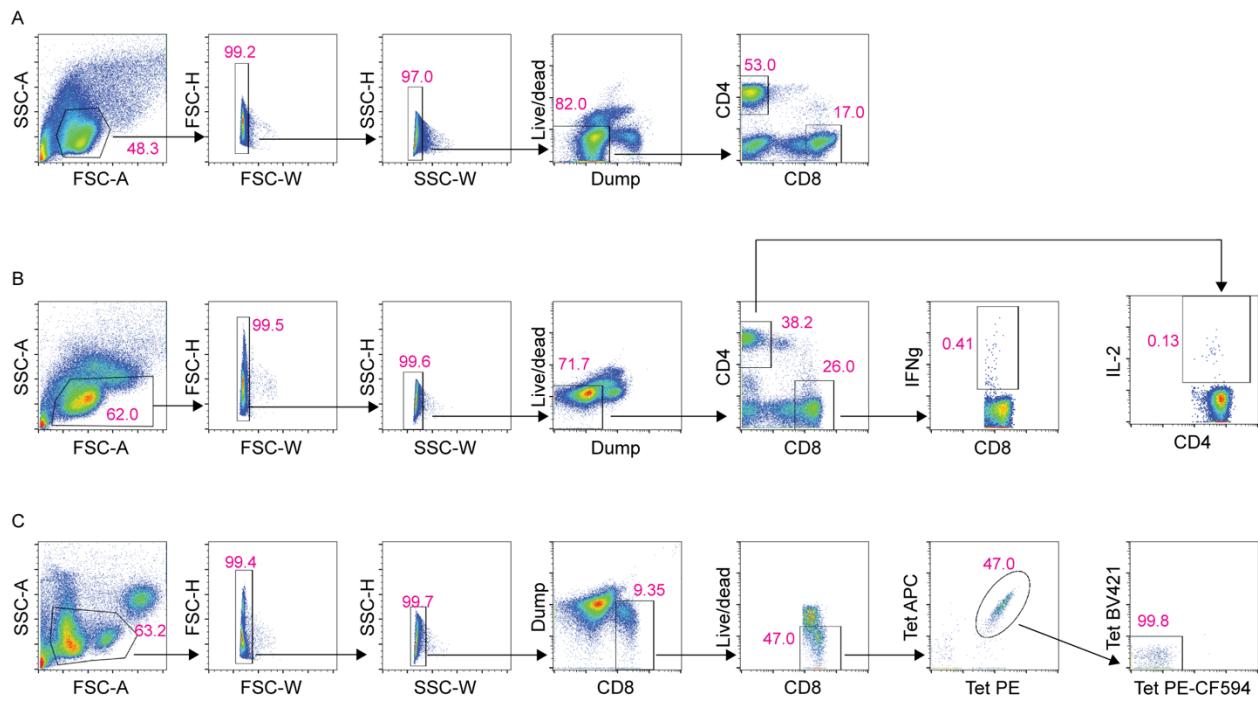
Supplemental Table 3. Overview of patient samples used for pMHC I tetramer analysis. HLA restriction refers to the relevant MHC I alleles used for tetramer staining. The tetramer panel refers to the panels outlined in Supplemental Table 2.



Supplemental Figure 1. Representative flow cytometry analysis for identification of immune cell subsets (**A**). Overview of immune cell subsets across all cohorts before and after vaccination: CD4+ T cells (**B**), CD8+ T cells (**C**), CD19+ B cells (**D**), CD14+ cells (**E**), CD14+ CD16+ cells (**F**), CD16+ cells (**G**). Comparisons of pre- and post-vaccination responses by multiple paired t-tests and comparisons of different patient cohorts by 2-way ANOVA with multiple comparisons.



Supplemental Figure 2. Analysis of vaccine-elicited spike-specific antibody responses in anti-CD20 mAb-treated MS patients. Post-vaccination net MFI of total spike IgG (**A, C**) and spike RBD IgG (**B, D**) by gender (**A-B**) and mRNA vaccine type (**C-D**), comparisons by Mann-Whitney. Net MFI of total spike IgG and spike RBD IgG versus last total IgG (**E-F**), %CD19+ B cells pre-vaccination (**G-H**), %CD19+ B cells post-vaccination (**I-J**), %CD19+ B cell change pre-vs post-vaccination (**K-L**), and interval (months) from last anti-CD20 mAb infusion to first vaccination (**M-N**), measured by simple linear regression and Spearman correlation.



Supplemental Figure 3. Gating overview for flow cytometry analysis. Representative gating strategies for AIM (**A**), ICS (**B**), and pMHC I tetramer enrichment (**C**). T cells were identified by live single cell lymphocytes that were dump antibody negative using the antibody panels from Supplemental Table 1. For tetramer enrichment, CD8+ T cells that were tetramer-positive in two fluorophores were subsequently gated on cells negative for the remaining two fluorophores to ensure specificity of tetramer binding.